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RESEARCH PAPER

Effects of cold plasma and ultrasound on reducing the allergenic potential of Brazil nuts

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Abstract

Background: the interest in edible seeds, such as Brazil nuts (*Bertholletia excelsa*), has grown due to their rich nutrient composition. The Brazil nut tree grows especially in the Amazon region, and the seeds hold economic and social importance. Despite Brazil nuts being nutritious, they are also associated with allergic reactions, due to proteins like 11S globulin (Ber e 2) and 2S albumin (Ber e 1) being the main allergens. In recent years, emerging technologies, such as high- power ultrasound and cold plasma, have been studied to improve food quality and safety by promoting alterations in protein properties. Objective: this study aims to compare these two physical methods on defatted Brazil nut cake, assessing their efficacy in reducing allergenic fractions and analyzing their proximate composition and amino acid profile. Methods: physical treatments with cold plasma and ultrasound were performed, followed by proximate composition and amino acid profile analyses. Results: indicate variations in ash, lipids, proteins, and carbohydrate contents between the cold plasma and ultrasound treatments. Ultrasound demonstrated a higher capacity to reduce lipid content and increase protein concentration, while cold plasma showed higher mineral retention. Concerning amino acid profile, cold plasma was the most effective treatment for rapidly releasing amino acids like cysteine and methionine. At the same time, ultrasound showed a more gradual and controlled action. Conclusion: neither method was sufficiently effective in reducing methionine, and cold plasma showed a temporary reduction in cysteine. The choice between the two methods depends on the specific objectives of each application.

Keywords

Allergenic protein, allergy, amino acid profiling, physical methods, proximal composition, vegetable protein

Introduction

Edible seeds, such as Brazil nuts (*Bertholletia excelsa* H.B.K), have received special attention as they are natural sources of vitamins, minerals, proteins, and essential fatty acids, contributing to a balanced diet for both humans and animals. Studies confirm that it is an oilseed with potential sources of bioactive compounds, which can provide significant health benefits through regular consumption (Alvarado-López et al. 2024).

In the Amazon region, Brazil nuts stand out as a food resource for the population and provide income for traditional communities, positively impacting the regional economy (Kainer et al. 2018). In this context, the Brazil nut species gains prominence. Some studies suggest that it can be used in human nutrition as a substitute for other

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plant-based foods due to its rich nutritional composition (Mazokopakis e Liontiris, 2018). Commonly known as the Pará nut, the Brazil nut tree reaches approximately 50 meters in height and up to two meters in diameter at the base. The fruit (pod) is a nearly spherical capsule (pyxidium) measuring 8 to 15 centimeters in diameter and containing 12 to 30 angular seeds (Barboza et al. 2019).

Brazil nut seeds can be consumed raw, cooked, or roasted, and are an excellent food source, serving as an ingredient for sweets, confectionery, and savory dishes. Just 2 to 3 seeds are enough to meet the daily intake of selenium, a vital mineral for endocrine and neurological functions, with anti-inflammatory properties (Thomson et al. 2008). These oilseeds are rich in proteins and lipids, contain no cholesterol, are good sources of fiber, and provide vitamins (A, E, B1, B2, and B3) and minerals such as calcium, magnesium, and selenium, among others (Schons et al. 2017). They also have considerable amounts of phosphorus and potassium, with low sodium content, thus benefiting the cardiovascular system (Baldoni et al. 2020). Studies on oilseeds show that the nutrient content of Brazil nuts from different regions varies greatly, which may reflect the different types of soils and environmental conditions where the trees are found (Vasquez-Rojas et al. 2021).

However, some proteins of Brazil nuts are associated with allergic reactions, allergies can be dangerous and may occur in highly atopic individuals of any age with a strong family history of atopy (Kuźmiński et al. 2021). The most important Brazil nut allergens are reserve proteins, such as 11S globulin (Ber e 2) and 2S albumin (Ber e 1) (Alcocer et al. 2021). Ber e 1 is associated with severe allergic reactions. It occurs in large quantities in Brazil nuts and is resistant to temperature and digestive processes (Rayes et al. 2016). 2S albumin is like the 2S albumin found in cotton, cocoa beans, sunflower seeds, rapeseed, walnuts, mustard seeds, and sesame seeds. Amino acid sequence comparisons of 2S albumin show that its composition is essentially rich in cysteine and methionine. Cysteine and methionine are sources of amino acids in high concentrations in Brazil nut proteins.

Conformational analysis of linear IgE-binding epitopes mapped on the surface of other allergenic proteins and Ber e 1 showed no structural homology, suggesting that the cross- reactions observed between, for example, peanuts and Brazil nuts may depend on other protein allergens, such as vicilin (Mendes et al. 2019). In patients with nut anaphylaxis, cross-reactions with hazelnuts and Brazil nuts have been reported (Kuźmiński et al. 2021).

Among the emerging technologies that could enhance food quality and safety is ultrasound processing. Ultrasound is applied to confer positive effects on food processing, such as improving mass transfer, preserving food, assisting thermal treatments, and analyzing texture. Sonication involves the formation of bubbles, which generate cavitation, followed by compression, high pressure, and heat, resulting in the breakdown of compounds (Bernardi et al. 2021). Cold plasma is a non-thermal technology that uses electrical discharges in a gas to generate a reactive medium capable of modifying the physical and chemical properties of foods. This treatment can alter protein structure by promoting the rupture of peptide bonds, modifying amino acid profiles, and increasing bioavailability. Recent studies indicate that protein treatment with cold plasma can increase protein solubility and improve the exposure of essential amino acids, such as lysine and leucine. Additionally, cold plasma can reduce the presence of allergenic and antinutritional compounds, expanding the use of vegetable by-products in human food. Unlike conventional processing techniques, cold plasma better preserves heat-sensitive amino acids, such as cysteine and tryptophan, preventing degradation. Given the above, this study aimed to compare physical methods in the defatted Brazil nut cake from the Amazon region to reduce the allergenic fraction through proximate composition analysis and amino acid profiling.

Materials and methods

One kilogram of Brazil nuts, harvested in March 2023, was purchased in Rio Preto da Eva/AM, Brazil. The oil extraction to obtain the defatted cake was conducted at the Food Composition and Toxicology Studies Center (NECTA) of the Federal University of Amazonas (UFAM) in Brazil. The oil was extracted through mechanical extraction using a hydraulic press. All samples were stored at −20 °C in airtight containers and protected from light and oxygen exposure until further analysis to prevent oxidation and degradation.

Physical-chemical analyses

Determinations of lipid, protein, total ash, and moisture content (%) were performed according to the analytical standards AOAC, with carbohydrates calculated by difference (AOAC 2016). All analyses were performed in triplicate.

Physical treatments cold plasma

The cold plasma treatment was conducted using a point-plane electrode reactor, which has a higher voltage arc distribution and a larger exposure area on the sample. The analysis was performed at the Environmental Sciences Laboratory of the Graduate Program in Environmental Sciences (PPGCA) at the University of Southern Santa Catarina (Unisul), Brazil. Protein inactivation was directly performed in the plasma within a solid reactor with a 400 mL capacity, with gas input and output (air and argon) operating at atmospheric pressure, working conditions of alternating current power of 17 kV and a current of 30 mA, and a plasma gas mixture (air and argon) at a 1:1 ratio with a gas flow rate of 3 L/min. Samples of 10 grams of defatted Brazil nut cake were added per experiment. The experiments were conducted in triplicate for 2, 5, and 10 minutes

based on preliminary trials aiming to observe both immediate and progressive effects of the treatment. To ensure treatment uniformity, samples were manually mixed between each replicate, and care was taken to maintain consistent positioning and distribution within the plasma chamber throughout the procedure.

High-power ultrasound

The defatted Brazil nut cake sample underwent ultrasound treatment, with the sample placed in a reactor, using an ultrasonic probe under the following conditions: 20 kHz (frequency), 50 W (power), 90 °C, for sonication times of 2, 5, and 10 minutes, all performed in triplicate. In addition, the ultrasound treatment was performed with an amplitude of 60%, using a probe with a 13 mm diameter. The treatment volume was 100 mL, and the estimated energy density was approximately 15 W/cm². Although the treatment reached a temperature of 90 °C, it is important to note that the process was initially non-thermal, and the temperature increase occurred as a result of extended sonication. The ultrasound technology application was conducted by an ultrasonic probe device from the Nano-Structured Polymers Laboratory (NANOPOL) at the Federal University of Amazonas (UFAM) in Brazil.

Statistical analysis

All analytical values were performed in three independent determinations. Data are represented as mean ± standard deviation. One-sided analysis of variance (ANOVA) for comparison, Tukey's test was used considering p-value < 0.05, using the BioStat 3.0 software.

Results

Nutrient composition

The nutrient composition of the defatted Brazil nut cake from the Amazon region, presented in Table 1, reveals notably high levels of fat, protein, ash, and moisture content compared to other high-nutritional-value products. These findings are consistent with previous studies (Alves et al. 2023) (Table 2), emphasizing the exceptional nutritional profile of Brazil nuts as a functional food source rich in essential macronutrients. Particularly, the defatted cake, as a by-product, maintains a significant nutritional value, making it a potential candidate for utilization in food formulations and nutritional supplements.

Table 1. Nutrients in Brazil nut protein isolate obtained by different methods.

Parameters (g/100 g -1)	Samples	
	Plasma	Ultrasound
Moisture	3.57 ± 0.27	3.76 ± 0.19
Ashes	5.76 ± 0.17	7.32 ± 0.21
Lipids	25.39 ± 0.28	22.72 ± 0.15
Proteins	18.78 ± 0.22	19.72 ± 0.11
Carbohydrates	46.48 ± 0.54	46.46 ± 0.14

All results are expressed as mean ± standard deviation of triplicates. Carbohydrates obtained by difference. Statistically significant differences between treatments at $p < 0.05$.

Physical treatments performance

Table 3 shows the analysis of amino acid profiles at different treatment times for cold plasma and ultrasound (US), revealing important variations in amino acid concentration between these methods. The behavior of each amino acid varies, reflecting the differences in the mechanisms of action of each technique and their effectiveness in modifying the protein composition. Table 4 shows the analysis of cysteine (Cys) and methionine (Met) in ultrasound and plasma treatments over different times (2, 5, and 10 minutes), revealing distinct patterns for these two sulfur-containing compounds.

Discussion

Proximate composition

The comparison of the proximate composition results between the Brazil nut cake treated with cold plasma and high-frequency ultrasound reveals significant variations in some nutritional components (greater protein availability), while others remain relatively stable. These differences can be attributed to the distinct ways each physical treatment affects the properties and molecular interactions of the present components.

Table 2. Comparison of Brazil nut nutrients.

Parameters (g/100 g -1)	Results		Alves et al. (2023)	
	Plasma	US	Brazil nut cake I	Brazil nut cake II
Moisture content	3.57 ± 0.27	3.76 ± 0.19	3.43 ± 0.10	5.58 ± 0.17
Ashes	5.76 ± 0.17	7.32 ± 0.21	10.72 ± 0.13	9.03 ± 0.13
Lipids	25.39 ± 0.28	22.72 ± 0.15	18.03 ± 0.11	25.56 ± 0.08
Proteins	18.78 ± 0.22	19.72 ± 0.11	19.31 ± 1.62	24.10 ± 0.68
Carbohydrates	46.48 ± 0.54	46.46 ± 0.14	48.51 ± 1.86	35.73 ± 0.53

All results are expressed as mean ± standard deviation from triplicates.
Values followed by different letters differ significantly ($p < 0.05$).

Table 3. Amino acid profile of Brazil nut cake treated with cold plasm and ultrasound.

Amino acids (g/100 g -1)	US (min)			Plasma (min)		
	2	5	10	2	5	10
Trip	0.07 ± 0.01	0.07 ± 0.01	0.11 ± 0.01	0.436 ± 0.02	0.453 ± 0.02	0.423 ± 0.02
Asp	0.02 ± 0.01	0.18 ± 0.01	0.33 ± 0.01	1.053 ± 0.13	1.1 ± 0.04	0.966 ± 0.07
Ser	0.11 ± 0.01	0.09a	0.16 ± 0.01	0.603 ± 0.06	0.603 ± 0.02	0.566 ± 0.05
Glu	0.43 ± 0.01	0.48 ± 0.01	0.07 ± 0.01	2.456 ± 0.22	2.57 ± 0.08	2.33 ± 0.15
Gly	0.07 ± 0.01	0.11 ± 0.01	0.16 ± 0.01	0.583 ± 0.05	0.576 ± 0.01	0.566 ± 0,04
Hys	0.09a	0.08 ± 0.01	0.10 ± 0.01	0.37 ± 0.02	0.396 ± 0.02	0.336 ± 0.05
Arg	0.06 ± 0.01	0.46 ± 0.01	0.06 ± 0.01	1.67 ± 0.09	1.783 ± 0.08	1.646 ± 0.07
Thr	0.33 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.366 ± 0.03	0.356 ± 0.01	0.336 ± 0.03
Ala	0.07 ± 0.01	0.09a	0.14 ± 0.01	0.44 ± 0.03	0.45 ± 0.01	0.416 ± 0.02
Pro	0.10 ± 0.01	0.13 ± 0.02	0.15 ± 0.01	0.573 ± 0.04	0.456 ± 0.02	0.423 ± 0.02
Tyr	0.07 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.436 ± 0.02	0.456 ± 0.02	0.423 ± 0.02
Val	0.11 ± 0.01	0.11 ± 0.01	0.16 ± 0.01	0.576 ± 0.05	0.586 ± 0.02	0.55 ± 0.03
Lys	0.07a	0.08 ± 0.01	0.10 ± 0.01	0.39 ± 0.04	0.416 ± 0.01	0.383 ± 0.02
Ile	0.07a	0.07 ± 0.01	0.11 ± 0.01	0.389 ± 0.03	0.393 ± 0.01	0.366 ± 0.01
Leu	0.17 ± 0.01	0.19 ± 0.01	0.26 ± 0.01	0.97 ± 0.07	1.0 ± 0.03	0.926 ± 0.05
Phe	0.11a	0.10 ± 0.01	0.16 ± 0.01	0.60 ± 0.03	0.62 ± 0.03	0.583 ± 0.03

All results are expressed as mean ± standard deviation from triplicates. a = not significant; other values show statistical difference at $p < 0.05$.

Table 4. Sulfur Amino Acids in Brazil Nut Cake Treated with Cold Plasma and Ultrasound.

Amino acids (g/100 g -1)	US (min)			% of reduction*	Plasma(min)			% of reduction*
	2	5	10		2	5	10	
Met	0.14 ± 0.01	0.17 ± 0.01	0.20 ± 0.01	92.2	0.643 ± 0.02	0.82 ± 0.12	0.883 ± 0.07	64.1
Cys	0.06 ± 0.01	0.08a	0.10 ± 0.01	84.6	0.643 ± 0.02	0.56 ± 0.08	0.62 ± 0.03	a

All results are expressed as mean ± standard deviation from triplicates. a = Not significant.
* concentration of free amino acids released (µmol/g) during physical treatments at different time intervals. Results are compared to time 0 (untreated control). Statistical analysis performed using ANOVA and Tukey's test ($p < 0.05$).

Concerning moisture content (3.57%) in the products treated with cold plasma (3.57 ± 0.27) and ultrasound (3.76 ± 0.19) is quite similar, with a small but significant difference. Ultrasound treatment shows a slight increase in moisture content. This behavior may be attributed to the effect of ultrasound on protein structure and the food matrix, which tends to retain more water due to pore formation or protein network disaggregation (Chen et al. 2022). On the other hand, cold plasma does not significantly alter water retention capacity, which is expected since its effect is more chemical than mechanical (Ashok et al. 2021).

The ash content, which reflects mineral content, shows a substantial difference between treatments. Cold plasma resulted in a lower ash value (5.76 ± 0.17) and ultrasound (7.32 ± 0.21). The difference may be related to the effect of ultrasound on the breakdown of protein and cell structures, facilitating the release and extraction of minerals from the food matrix (Hussain et al. 2023). While cold plasma can alter the surface composition of components, it has less impact on mineral extraction than ultrasound.

Lipids show a significant reduction in content after ultrasound treatment (22.72 ± 0.25) compared to cold plasma (25.39 ± 0.28). Ultrasound, known for its cavitation capability and intense mechanical forces, can promote lipid release, facilitating their extraction and consequently reducing the remaining lipid content in the defatted cake. Cold plasma, on the other hand, has a smaller mechanical effect and therefore less impact on lipid reduction, maintaining a higher content compared to ultrasound.

There was a slight difference in proteins between treatments, with ultrasound showing a slightly higher value (19.72 ± 0.11) than cold plasma (18.78 ± 0.22). This difference, although small, may be attributed to ultrasounds' ability to disaggregate proteins and make their fractions more accessible and soluble (Akharume et al. 2021). Ultrasound may enhance protein extraction or at least facilitate the measurement of soluble proteins in subsequent analyses. Cold plasma, on the other hand, may cause chemical modifications to proteins, such as oxidation, which can slightly influence the detectable protein content without significant impacts (Misra et al. 2016). Carbohydrates show nearly identical values between cold plasma (46.48± 0.54) and ultrasound (46.46 ± 0.14) treatments. This suggests that none of the treatments significantly alters the carbohydrate fraction of Brazil nut cake. This is expected, as both ultrasound and cold plasma primarily affect protein, lipid, and mineral fractions, while carbohydrates, being structurally stable and less susceptible to cavitation or mild oxidation, remain mostly unchanged.

Physical treatments

For most amino acids, plasma treatment induces higher concentrations than ultrasound. The concentrations of tryptophan (Trip) moderately increase with ultrasound treatment, but plasma shows significantly higher values

already at 2 minutes (0.436 g/100 g), maintaining elevated levels up to 10 minutes (0.423 g/100 g). According to Zhang and Mittal (2018), this behavior suggests that plasma treatment may be more efficient in degrading or releasing tryptophan from the protein matrix. It is noteworthy in aspartic acid (Asp) that there is a difference between treatments, with plasma showing substantially higher concentrations (1.053 g/100 g at 2 minutes) compared to ultrasound, where the increase is more gradual and peaks at a lower concentration of 0.33 g/100 g at 10 minutes. This suggests a more efficient action of plasma in hydrolysis or protein modification that releases aspartate.

Some amino acids exhibit stabilization behavior over time, both in ultrasound and plasma treatments. Serine (Ser) in ultrasound treatment shows slight variation between 0.09 g/100 g and 0.16 g/100 g over the time intervals. In plasma treatment, serine maintains stability at concentrations of approximately 0.603 g/100 g for up to 10 minutes. This consistency may indicate that serine is not highly affected by the two methods after a certain treatment time. Similarly, the concentration of glycine (Gly) also shows stability, especially in plasma treatment, around 0.566 g/100 g. In ultrasound, the increase is slight and continuous, reaching 0.16 g/100 g at 10 minutes. This profile reflects a lower susceptibility of glycine to variations in treatments. The impact of time is more evident in some amino acids, particularly in ultrasound treatment. The concentration of glutamic acid (Glu) drops sharply between 5 and 10 minutes (from 0.48 to 0.07 g/100 g), indicating possible rapid degradation or modification of glutamate. In contrast, in plasma, the values remain consistently high, peaking at 2.57 g/100 g at 5 minutes, suggesting that plasma favors the preservation or continuous release of this amino acid. In ultrasound, leucine (Leu) increased continuously (from 0.17 to 0.26 g/100 g), indicating that prolonged ultrasound promotes the release of this amino acid. However, in plasma, the increase is more modest and stabilizes after 5 minutes, which may suggest that leucine release reaches a saturation point more quickly with plasma.

Studies indicate that ultrasound can modify the biochemical properties of proteins, such as their solubility and conformation, making them more susceptible to degradation or making the epitopes (regions that bind to IgE) less accessible. By breaking intermolecular interactions in proteins, ultrasound can help reduce the IgE-binding capacity of these proteins, as observed in studies with other nuts and legumes. Additionally, when combined with enzymatic treatments, ultrasound can be even more effective in reducing IgE-binding capacity, showing promising options for hypoallergenic foods development (Cuadrado et al. 2023).

This approach represents a significant advancement in the production of foods that can be consumed more safely by allergic individuals while maintaining the nutritional benefits of nuts. However, further studies are needed to assess the clinical efficacy of these methods, as reduced IgE reactivity in the lab does not always translate into an actual reduction in allergic reactions in vivo (Cuadrado et al. 2023).

Tobajas et al. (2023) investigate the influence of thermal and ultrasound treatments on the denaturation and allergenic potential of the Pru p 3 protein, a major allergen found in peaches, which shares characteristics with allergens in nuts like Brazil nuts. Although the study focuses on peaches, it provides valuable insights applicable to Brazil nuts, particularly on the stability of allergenic proteins and the potential of non-thermal processing technologies to modify their allergenicity.

In the context of Brazil nuts, where proteins such as 2S albumin (Ber e 1) are the main allergens, ultrasound could disrupt the allergenic protein aggregates, making them more accessible to degradation or less capable of binding to IgE, which triggers allergic reactions. The modifications in secondary structures (α-helix, β-sheet, etc.) and fluorescence spectra observed in the study indicate that ultrasound causes unfolding and rearrangement of proteins, which may decrease their ability to cause allergic reactions (Zhao et al. 2021).

Although the article primarily deals with walnuts, the mechanisms discussed, such as protein structural modification through cavitation and microstreaming, can be applied to reducing allergenic potential in Brazil nuts. By changing the protein structure, HIU can potentially expose the epitopes less, reducing IgE binding, thus lowering the risk of allergic responses. However, the study suggests that this reduction in allergenicity would need to be confirmed with specific allergenicity assays relevant to Brazil nuts (Zhao et al. 2021).

Differences between plasma and ultrasound

Overall, plasma appears to be more efficient in the release of amino acids such as aspartic acid, glutamic acid, and arginine. The concentration of these amino acids is consistently higher in plasma treatment, possibly due to greater protein breakdown in the sample. Although ultrasound also promotes amino acid release, it seems to do so in a more gradual manner and with less intensity than plasma. This may be advantageous in cases where more controlled release is desired but less effective for rapid acquisition of free amino acids (Chemat et al. 2010). In the plasma treatment, there is a high initial concentration of cysteine (0.643 g/100 g at 2 minutes), followed by a slight drop at 5 minutes (0.56 g/100 g), and then another rise at 10 minutes (0.62 g/100 g). This profile suggests that plasma promotes a rapid and intense release initially, with a slight fluctuation over time. Methionine also shows a gradual increase in the ultrasound treatment, starting at 0.14 g/100 g at 2 minutes and reaching 0.20 g/100 g at 10 minutes. Ultrasound appears to have a mild but consistent effect on methionine release. Plasma treatment, like cysteine, induces a significant increase in methionine concentration. At 2 minutes, the concentration is 0.643 g/100 g, rising to 0.82 g/100 g at 5 minutes, and reaching 0.883 g/100 g at 10 minutes.

This behavior suggests that plasma is highly efficient in releasing methionine, with a faster and more pronounced response than ultrasound. Plasma was the most effective treatment for reducing cysteine, as it caused a temporary decrease in its concentration at 5 minutes. The concentration of cysteine increases gradually over time. At 2 minutes, the concentration is 0.06 g/100 g, reaching 0.10 g/100 g after 10 minutes. This increase indicates that ultrasound may facilitate the release or modification of cysteine, although more slowly and less intensely. Ultrasound, on the other hand, was ineffective in reducing it, promoting a continuous increase in the amino acid. Both treatments failed to reduce methionine, with plasma causing an even greater increase than ultrasound. Therefore, if methionine reduction is necessary, the shortest treatment time (2 minutes) should be used.

Ultrasound high-intensity treatment alters the molecular structure of proteins by promoting the unfolding of α-helices and β-turns while increasing the content of random coil structures. In the case of Brazil nut proteins, such as 2S albumin (Ber e 1), known for their high stability, these structural changes could reduce the exposure of epitopes that bind to IgE antibodies, responsible for allergic reactions (Tian et al. 2024).

Ultrasound does not break the peptide bonds of proteins but significantly affects their functional properties, such as solubility and their foaming and emulsifying capacity. For Brazil nuts, these modifications may reduce the IgE-binding capacity, decreasing the risk of triggering allergic reactions (Tian et al. 2024). Furthermore, ultrasound increases the exposure of sulfhydryl (SH) groups in proteins, which could indicate partial denaturation of allergenic proteins, making them less likely to trigger an immune response.

Ultrasound, which is also explored as a non-thermal processing option, shows some promise in denaturing proteins, but its effect on allergenicity is minimal. The study suggests that more innovative combinations of processing techniques might be necessary to significantly lower the allergenic potential of proteins such as those found in Brazil nuts (Tobajas et al. 2023).

The study demonstrated a reduction in the allergenic fraction after physical treatment.. This observation aligns with recent advances in the assessment of food protein allergenicity, which emphasize the importance of integrated strategies to predict the allergenic risk of novel protein sources.

According to the Mills et al. (2024), allergenicity assessment of food proteins should incorporate integrated approaches, including in silico, in vitro, and, when necessary, in vivo testing, following a "weight of evidence" framework to ensure consumer safety. This approach is further supported by Fernandez et al. (2020), who highlight the need to improve predictive criteria for allergenicity, particularly in proteins derived from novel or processed foods, such as those treated by ultrasound.

Additionally, the review conducted by the Food Standards Agency (2022) emphasizes that molecular methods, such as ELISA and Western blot, remain reliable tools for detecting allergenic epitopes, especially when combined with structural and functional analyses. The absence of recognized epitopes after treatment observed in this study may indicate a potential allergenicity-reducing effect, although further in vivo and clinical testing is required to confirm this finding.

Conclusion

A comparison of physical-chemical methods was carried out to evaluate the amino acid profile from Brazil nuts defatted cake. Plasma treatment appears to be more effective at increasing the concentration of several amino acids in shorter periods, while ultrasound promotes more gradual and less intense changes. The impact of time on the amino acid profile is more pronounced in the ultrasound treatment, where some concentrations continue to increase or decrease after 10 minutes, while plasma tends to stabilize concentrations after 5 minutes. These results suggest that the choice of treatment method and timing will depend on specific objectives related to modifying the amino acid profile in a sample.

Plasma treatment was the most effective for the rapid release of amino acids such as cysteine and methionine, while ultrasound demonstrated a more gradual and controlled action. However, both methods were insufficient for reducing methionine, and plasma caused a temporary reduction in cysteine. The choice between the two methods depends on the specific goals of each application. Further studies on the possible modification of the amino acid profile should be encouraged to achieve changes in the protein fraction of interest for use in food technology.

In summary, while ultrasound and thermal treatments can cause structural changes in allergenic proteins, these methods alone are insufficient to ensure a reduction in allergenicity. Further research is needed to develop effective food processing methods to mitigate allergic reactions in sensitive individuals moving towards safer food.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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